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Experimental studies on the taxonomic status of some members of the *Onychiurus armatus* species group

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INTRODUCTION

On the basis of material collected from the Swiss Alps, GISIN (1952 a) expressed the opinion that the species *Onychiurus armatus* (Tullberg, 1869) includes a diversity of forms of specific status ; twelve were named by GISIN (1952 a) and later a thirteenth (1952 b). GISIN (1960) records 38 species which are contained within the *Onychiurus armatus* species group. The main characters used in the separation of the species within the group are :

i. The location and number of pseudocelli on different segments of the body.

ii. The arrangement of setae on the tergites of the first thoracic segment and those of abdominal segments five and six. Other minor characters involved are the relative lengths of the anal spines and the presence or absence of an inner tooth on the claw.

The division of the *Onychiurus armatus* species group has been criticised by STACH (1954) and by BÖDVARSSON (1959). The former author considers most of GISIN's species to be « insignificant ecological or local modifications » and the latter author writes as follows: « As long as the biological conditions of the animals and the nature of the variations have not been studied experimentally, it seems most appropriate to regard these forms as infrasubspecific and thus not to submit them to purely taxonomic treatment ». In the pre-

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sent paper GISIN's criteria have been assessed experimentally in four species of the group, and found to be valid; differences in the breeding biology and ecology have also been found.

The four species selected for study can be divided into two groups ecologically. *Onychiurus procampatus* Gisin 1956 and *Onychiurus tricampatus* Gisin 1956 are true soil Collembola, both occurring at depth in mull soils, whilst *Onychiurus latus* Gisin 1956 and *Onychiurus fimatus* Gisin 1952 are found in more acidic conditions and are generally restricted to the litter layer. *Onychiurus latus* is bright yellow whereas the other three species are colourless.

Soil samples collected in the field were brought into the laboratory and the Collembola extracted using a large Tullgren funnel. The Collembola fell into a vessel containing water, from the surface of which they were removed and introduced into culture jars of the type described by GOTO (1961). Individual Collembola and eggs were kept in $2 \times 3/4$ inch specimen tubes filled to within 1/2 inch of the top with a plaster of Paris/charcoal mixture, and covered by a 3/4 inch diameter cover slip retained by a film of vaseline. The cultures were maintained at 15° C.

The results of breeding in the laboratory.

Cultures of each of the four species were set up and eggs were removed within a day of laying. In all cases the individuals which hatched from the eggs were, on reaching maturity, found to be identical with the parent stock in both pseudocellar formula and chaetotaxy. The typical yellow colouration of *Onychiurus latus* was present in first instar individuals of that species.

Some aspects of the chaetotaxy of different instars of *Onychiurus procampatus*, *Onychiurus tricampatus* and *Onychiurus latus* have been described elsewhere (HALE in press) and a full analysis of the chaetotaxy and pseudocellar formulae of different instars of these species, *Onychiurus fimatus* and others of the *Onychiurus armatus* species group is at present in the course of preparation.

No intermediate forms resulted from the laboratory cultures but the relationship existing between *Onychiurus procampatus* and *Onychiurus tricampatus* requires explanation.

The relationship between *Onychiurus procampatus* and *Onychiurus tricampatus*.

In twelve different areas samples were taken which contained both *Onychiurus procampatus* and *Onychiurus tricampatus*; in no locality was either species found alone. Whilst little difficulty was experienced in distinguishing between the two species when individuals were mounted on microscope slides, initially it was found impossible to separate them in life. Subsequently it was found that all the largest individuals (average head capsule length 343 microns) were *Onychiurus procampatus*. Detailed examin-

ation of 2,097 individuals of these two species collected from a single limestone outcrop on the Moor House Nature Conservancy Reserve, Westmorland, revealed that 362 were female *Onychiurus procampatus*, 210 were male *Onychiurus tricampatus* and 216 were female *Onychiurus tricampatus;* in 1,309 individuals the sex was not determined because they were either very young specimens or mounted in such a way as to obscure the details of the genital plate. Males of *Onychiurus procampatus* were absent; this proved to be the case in ten other areas from which specimens were collected (Table 1).

TABLE I

Numbers of Onychiurus procampatus **and** Onychiurus tricampatus **collected in various sampling areas**

Locality	*Onychiurus procampatus*		*Onychiurus tricampatus*	
	Male	Female	Male	Female
1. Moor House, Westmorland, Limestone, House Field	0	362	210	216
2. Moor House, Westmorland, Limestone, Green Hole	0	64	27	49
3. Moor House, Westmorland, Limestone, Milburn Beck	0	10	28	32
4. Moor House, Westmorland, Alluvium, Trout Beck	0	34	13	15
5. Cassop Vale, Co. Durham, Limestone	0	174	32	56
6. Melmerby, Cumberland, Limestone	32	68	2	3
7. Hartside, Cumberland, Limestone	0	14	8	13
8. Malham, Yorkshire, Limestone	0	176	33	42
9. Bowes Moor, Yorkshire, Limestone	0	11	3	13
10. Milngavie, Dunbartonshire. Sample from S. Milne	0	12	1	0
11. Mewslade Bay, Gower, Glamorgan. Sample from A. Macfadyen & I. Healey.	0	25	7	21
12. Vorso, Denmark. Sample from H. Gisin.	0	4	2	3

Individuals of head length greater than 300 microns (*Onychiurus procampatus* females) were placed in culture jars, twenty individuals to each jar. No eggs were laid despite the fact that parthenogenesis was to be expected in the absence of males in the field. In ten culture jars containing only *Onychiurus procampatus* females no eggs were laid, whereas in over thirty jars containing both *Onychiurus procampatus* and *Onychiurus tricampatus* both species laid eggs. The presence of males of *Onychiurus tricampatus* was ascertained after the production of eggs in the cultures by mounting and examining all individuals. In order to ensure that this situation was not a result of collecting Collembola for the cultures at different times, samples were taken from Malham, Yorkshire, Cassop, County Durham and Moor House, Westmorland, and pairs of cultures set up from individuals collected on the same day. One culture jar of each pair contained only *Onychiurus procampatus,* whilst the other of the pair contained a mixture of both species. No eggs were laid in

any of the six cultures (two from each area) containing only adult *Onychiurus procampatus* females, but eggs of this species were laid in all six cultures containing both species.

The possibility of social facilitation to egg laying was considered and large cultures of more than fifty individuals of *Onychiurus procampatus* were set up; in addition to this, groups of ten individuals of this species were placed in cultures of *Onychiurus furcifer* (Börner 1912) where eggs were being laid. In all cases *Onychiurus procampatus* failed to produce eggs. It thus appears to be necessary for males of *Onychiurus tricampatus* to be present before female *Onychiurus procampatus* will lay eggs.

Isolation of the eggs of *Onychiurus procampatus* and subsequent examination on hatching showed that in all the cases examined (over 150) the hatched individuals were referable to *Onychiurus procampatus* and not to *Onychiurus tricampatus*; that is to say all were females, all had the morphological characters of *Onychiurus procampatus*, and all developed into large individuals with an average head capsule length of 340 microns in the adult stage. All the eggs laid by *Onychiurus tricampatus* females (over 200) hatched into individuals referable to *Onychiurus tricampatus*; both males and females were present, and all developed into adult individuals with an average head capsule length of 260 microns. The eggs of the two species were distinguishable by their different sizes (see later).

Whilst eggs were not produced by *Onychiurus procampatus* females in the absence of *Onychiurus tricampatus* males, even when male *Onychiurus tricampatus* were present, a percentage of the eggs failed to develop. 43 % of all eggs laid by *Onychiurus procampatus* did not develop, that is the chorion did not split, compared with 26 % of the eggs of *Onychiurus tricampatus*. The fact that 43 % of the eggs failed to develop in circumstances identical to those in which the other 57 % developed suggests infertility; if this is in fact so, then the eggs of *Onychiurus procampatus* must be fertilised by *Onychiurus tricampatus* males.

The status of the large female *Onychiurus procampatus*.

Large female *Onychiurus procampatus* always breed true in cultures. Since in all but one of the areas examined this species is entirely of females, which always give rise to females, apparently breeding is a form of parthenogenesis. However, it appears that the presence of *Onychiurus tricampatus* males is necessary in order that fertile eggs may be produced; true fertilisation does not, however, appear to take place, as *Onychiurus procampatus* has bred true for three generations in culture without the appearance of any forms intermediate between this species and *Onychiurus tricampatus*. For this reason *Onychiurus procampatus* and *Onychiurus tricampatus* are not considered to be polymorphic forms of the same species by the present writer, although the possible action of lethal factors cannot entirely be ruled out to account for the lack of males in *Onychiurus procampatus*.

It seems possible that this is a case similar to that occurring in the grain beetles of the Genus *Ptinus*. Here *Ptinus latro* Fab. 1775 occurs always as females which are normally « fertilised » by the males of *Ptinus hirtellus*

Sturm 1837, although males of other species can bring about « fertilisation » (MOORE, WOODROFFE and SANDERSON, 1956). Apparently entry of the sperm into the egg of *Ptinus latro* stimulates development mechanically, and there is no fusion of the nuclei or exchange of genetic material.

It appears probable that *Onychiurus procampatus* and *Onychiurus tricampatus* are good species since there is apparently no transfer of genetic material and breeding is probably a form of thelytokous parthenogenesis. The occurrence together of the two species in all the areas examined can be explained by the apparent necessity of the presence of *Onychiurus tricampatus* males before *Onychiurus procampatus* will lay eggs that eventually develop.

Normal sexual reproduction in *Onychiurus procampatus*.

In the material furnishing the types of *Onychiurus procampatus* taken by Dr. H. GISIN from Engelalp, Bernese Alps, Switzerland, males were present. In the adult stage these were the size of the largest *Onychiurus tricampatus* (average head capsule length 264 microns in the adult stage), and much smaller than the adult *Onychiurus procampatus* (large females) taken in the North of England. At Melmerby, Cumberland, adult *Onychiurus procampatus*, both males and females, were collected which were identical in head capsule length with adult *Onychiurus tricampatus* found in the same samples. Cultures of the small *Onychiurus procampatus* were set up and it was found to reproduce sexually, laying small eggs identical in size with those of *Onychiurus tricampatus*. The first instars were also identical in head capsule size with *Onychiurus tricampatus*, but differed consistently in the pseudocellar formula and in the chaetotaxy. These bred true and the males were capable of stimulating development in the eggs of the large female *Onychiurus procampatus*. Thus two types of adult females occur in *Onychiurus procampatus*, a large apparently parthenogenetic form and a small sexually reproducing form. The fact that *Onychiurus tricampatus* was present in the same samples and no intermediates were found between the sexually reproducing individuals of *Onychiurus procampatus* and *Onychiurus tricampatus* suggests that these are good species.

Dimorphism in females of other members of the *Onychiurus armatus* species group.

Examination of over 200 individuals of *Onychiurus fimatus* collected in Durham showed that whilst all adults were similar from the point of view of pseudocellar formula and chaetotaxy, two different sizes of mature females occurred, equal in head capsule length to the two forms of *Onychiurus procampatus*; small males were also present but there were no large males. Choudhuri (1958 and 1961) reports that in *Onychiurus fimatus* there are seven instars (size groupings), one more than in *Onychiurus procampatus* and *Onychiurus tricampatus* (HALE in press). Only six groupings, with a Dyar's increment factor of 1.15 were found in *Onychiurus fimatus* from Durham.

The increment factor was identical with that found by CHOUDHURI. Plotting all individuals (large and small) of the sample, eight groupings of head capsule size occur. The probable reason for lack of agreement between the results of this work and those of CHOUDHURI lies in the occurrence of two types of female which were unrecognised by the latter author. Examination of material of *Onychiurus fimatus* identified by CHOUDHURI has been carried out, and the presence of the two size groupings observed in it. It thus appears that in *Onychiurus fimatus* there is a similar dimorphism to that occurring in *Onychiurus procampatus*, and possibly parthenogenetic and sexually reproducing forms exist.

Examination of *Onychiurus quadriocellatus* Gisin 1947, taken from the Swiss Jura, showed a similar phenomenon with two sizes of females and only small males, and GISIN (pers. comm.) has now observed this in the same species from another habitat.

Differences in breeding biology.

During the course of culturing the four species of *Onychiurus* various differences in the breeding biology were observed (HALE in press); these are as follows:

1. The eggs of *Onychiurus latus* and the large form of *Onychiurus procampatus* were the same size on laying (230 microns in diameter), and larger than the eggs of the small form of *Onychiurus procampatus*, the small form of *Onychiurus fimatus* and *Onychiurus tricampatus* (170 microns in diameter on laying).

2. Significant differences were found in the sizes of egg batches produced by large female *Onychiurus procampatus*, *Onychiurus tricampatus* and *Onichiurus latus*, but no differences occurred in the development times at constant temperatures.

3. Egg laying in the field was restricted to a spring period in *Onychiurus latus*, a hemiedaphic form, but continued through summer into autumn in *Onychiurus procampatus* (large females) and *Onychiurus tricampatus*.

4. Significant differences were found in the duration of equivalent instars in *Onychiurus procampatus*, *Onychiurus tricampatus* and *Onychiurus latus*.

5. In *Onychiurus latus* there were seven instars before maximum head capsule length was reached, and in the other three species only six instars. In *Onychiurus procampatus* first instar individuals hatching from large eggs (laid by large females) had a head capsule length equivalent to that of third instar individuals hatching from small eggs (laid by small females).

Lack of recognition of dimorphism in the females would result in eight size groupings in field populations (instead of six) and thus, apparently, eight instars in both *Onychiurus procampatus* and *Onychiurus fimatus*. Since the chaetotaxy and pseudocellar formula are identical in the same instars of dimorphic forms of the same species, and since there is a size difference in these forms, this has probably given rise to the claim that individuals of similar size possess different pseudocellar formulae and chaetotaxy. Again,

the adults of different species within the *Onychiurus armatus* species group may vary in size ; here, again, comparison of similar sized individuals taken in the same samples can easily lead to confusion.

Differences in ecology.

It has already been pointed out that *Onychiurus procampatus* and *Onychiurus tricampatus* are true soil Collembola, occurring at depth in mull soils, whereas *Onychiurus latus* is restricted to more acidic conditions. On the Moor House Reserve, *Onychiurus procampatus* (parthenogenetic females) and *Onychiurus tricampatus* occurred together in the same sample units $\frac{1}{1000}$ m^2 $\times$ 6 cm deep, taken from both limestone and alluvial soils. Comparison of the total numbers of these two species in separate (3 cm) layers indicates a significant difference in their depth distribution (Table 2). It is possible that

TABLE II

Depth distribution of all Onychiurus procampatus **and** Onychiurus tricampatus **from Limestone grassland, February 1960 to December 1961**

Depth (cm)	*O. procampatus*	*O. tricampatus*	Total
0-3	738	618	1,356
3-6	113	352	465
TOTAL	851	970	1,821

$\chi^2 = 126.2$ d. f. = 1 $P < 0.001$

this difference is merely a reflecton of the differing sizes of the two species, where the larger *Onychiurus procampatus* is less able to penetrate into the soil. It has been pointed out that the last four instars of *Onychiurus tricampatus* are equivalent in head capsule length to the first four instars of *Onychiurus procampatus* (only large females occur at Moor House) ; the dimensions of the bodies of these four instars are also similar. Thus, comparison of

TABLE III

Depth distribution of individuals of similar size in Onychiurus procampatus **and** Onychiurus tricampatus **from Limestone grassland. February 1960 to December 1961**

Depth (cm)	*O. procampatus*	*O. tricampatus*	Total
0-3	493	478	971
3-6	68	264	332
TOTAL	561	742	1,303

$\chi^2 = 94.5$ d. f. = 1 $P < 0.001$

the depth distribution of the four instars of equal dimensions will indicate whether or not the vertical distribution is a function of the different sizes of the two species. The data in Table 3 show that there is a significant difference between the vertical distribution of individuals of equivalent size, and thus a factor other than size must affect the vertical distribution of the two species in question. Comparison of the vertical distribution of the individual instars is made in Table 4. It can be seen that there is a significant difference between the vertical distribution of the two species during the first five instars, *Onychiurus tricampatus* tending to occur deeper than *Onychiurus procampatus*.

TABLE IV

Comparison of the numbers of different instars of Onychiurus procampatus **and** Onychiurus tricampatus **in the 0-3 cm and 3-6 cm layers. Limestone grassland, February 1960 to December 1961**

Species	Layer	Instar					
		1	2	3	4	5	6
Onychiurus	0-3 cm	46	181	110	151	140	90
procampatus	3-6 cm	11	30	10	17	25	20
Onychiurus	0-3 cm	23	117	110	173	139	56
tricampatus	3-6 cm	20	68	66	91	83	24
χ^2		8.5	26.9	39.3	32.5	23.3	3.6
P for 1 degree of freedom		< 0.005	< 0.001	< 0.001	< 0.001	< 0.001	> 0.05

Consistency of the pseudocellar formula.

The present study has shown that the morphological criteria on which Gisin (1952 a and b) broke down the *Onychiurus armatus* species group are supported by differences in ecology and breeding biology in the four species discussed in this paper. Bödvarsson (1959) states that the various species within the *Onychiurus armatus* species group are connected by individuals which are intermediate in morphological characters. Examples have not been found which support this contention and it is likely that this author has not taken into account variation in the pseudocellar formula and chaetotaxy in various instars, and differences resulting from the variation in size in the dimorphic forms. If Gisin's taxonomic criteria are applied to individuals after the fourth instar in the species concerned in this paper, complete separation can be made. Occasionally the pseudocelli of one side of an individual are duplicated or omitted in places, but provided that both sides are examined carefully this can usually be recognised. Table 5 shows a comparison of the pseudocellar formulae of the four species of *Onychiurus* with which this paper is concerned. Apparently the number and position of the pseudocelli are not affected by the environment in the same way that certain characters are affected by temperature in other Genera, for example *Hypogastrura* (Cassagnau, 1955). In thoses species studied the criteria of Gisin (1952) are valid, since they are consistent from generation to generation and

true intermediates do not exist. The taxonomy of the group is, however, complicated by dimorphism in females of some species, and apparently by a form of thelytokous parthenogenesis which requires further study.

TABLE V

The pseudocellar formulae in different instars of four species of the Onychiurus armatus **species group**

Instar	*Onychiurus procampatus*	*Onychiurus tricampatus*	*Onychiurus latus*	*Onychiurus fimatus*
1	32/022/33332	32/023/33332	32/022/33332	22/022/33332
2	33/022/3333 or 42 or 3	32/02 or 33/33332 or 3	33/022/3333 or 42 or 3	32 or 3/022/33332 or 3
3	33/022/33343	33/02 or 33/33343	33/022/33343	33/022/33333
4	33/022/33343	33/02 or 33/33343	33/022/33343	33/022/33333
5	33/022/33343	33/02 or 33/33343	33/022/33343	33/022/33333
6	33/022/33343	33/02 or 33/33343	33/022/33343	33/022/33333
7	—	—	33/022/33343	—

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Summary.

1. *Onychiurus procampatus, Onychiurus tricampatus, Onychiurus latus* and *Onychiurus fimatus* bred true in culture and the resulting adults were identical with the parent stock.

2. *Onychiurus procampatus* and *Onychiurus fimatus* possess dimorphic females. In *Onychiurus procampatus* large females (average head capsule length 343 microns) appear to be parthenogenetic; these always lay large eggs (230 microns in diameter on laying) which give rise to large females. Small females (average head capsule length 264 microns) lay small eggs (170 microns in diameter on laying) which give rise to small males and females. Large females would not lay in isolation but only if males of *Onychiurus procampatus* or *Onychiurus tricampatus* were present. The possibility of gynogenesis or some similar phenomenon is considered.

3. Differences in egg batch sizes, the number of instars to reach maturity, the period of egg laying in the field and differences in vertical distribution (*Onychiurus tricampatus* occurs deeper than *Onychiurus procampatus*) are offered as additional evidence in support of Gisin's division of the *Onychiurus armatus* species group, which is considered to be valid by the present author.

References

BÖDVARSSON, H. 1959. Studien uber die Variation einiger systematischen Charaktere bei *Onychiurus armatus* (Tullberg 1869) (Collembola). Opusc. ent. 24 : 225-245.

CASSAGNAU, P. 1955. L'influence de la température sur la morphologie d'*Hypogastrura purpurascens* (Lubbock), Collembole Poduromorphe. *C. R. Acad. Sci.* 240 : 1483-1485.

CHOUDHURI, D. K. 1958. Some aspects of the Biology and Systematics of some species of *Onychiurus* (Collembola). Thesis : University of London. 1961. Temperature and its effect on three species of the genus *Onychiurus* Collembola. *Proc. Zoo. Soc.* (Bengal). 16 : 97-117.

GISIN, H. 1952 a. Notes sur les Collemboles, avec démembrement des *Onychiurus armatus, ambulans* et *fimetarius* auctorum. Mitt. Schweiz. Ent. Ges. 25 : 1-22.

1952 b. *Onychiurus vanderdrifti* n. sp. (Collembola). *Entomol. Berichten,* 14 : 61.

1960. *Collembolenfauna Europas.* Geneva.

GOTO, H. E. 1961. Simple techniques for the rearing of Collembola and a note on the use of a fungistatic substance in the cultures. *Ent. mon. Mag.,* 96 : 138-140.

MOORE, B. P. Woodroffe, G. E. and Sanderson, A. R. 1956. Polymorphism and parthenogenesis in a Ptinid beetle. *Nature,* 177 : 847-848.

STACH, J. 1954. The Apterygotan Fauna of Poland in Relation to the World Fauna of this Group of Insects. Family Onychiuridae. *Polska Akademia Nauk.,* Krakow.